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PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Twine *et al.*	CONFIRMATION NO.:	3640
APPLICATION NO.:	10/717,597	GROUP NO.:	1639
FILING DATE:	November 21, 2003	EXAMINER:	Liu, Sue Xu
TITLE:	Methods for Diagnosing RCC and Other Solid Tumors		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MICHAEL E. BURCZYNSKI UNDER 37 C.F.R. § 1.132

Sir:

I, Michael E. Burczynski, hereby declare as follows:

1. I am a coinventor of the above-referenced patent application.

2. I hold a Ph.D. in Pharmacology from the University of Pennsylvania. I have studied gene expression profiling and pharmacogenomics since January of 2000 as a post-doctoral research fellow for Johnson and Johnson from January of 2000 through March of 2001; as a senior research scientist in Clinical Pharmacogenomics for Wyeth Research from March of 2001 through December of 2004; and as a Principal Scientist and Laboratory Head for the Pharmacogenomic Biomarkers Biomarker Laboratory since December of 2004. I am a lecturer in the Department of Pharmacology at the University of Pennsylvania School of Medicine. I author peer-reviewed articles and give presentations relating to gene expression profiling and pharmacogenomics.

3. I have been asked whether a person of ordinary skill in the art would have expected most genes involved with NFκB signaling to be differentially expressed in peripheral blood mononuclear cells (PBMCs) of patients having a non-blood disease such as RCC as compared to PBMCs of disease-free humans.

4. In my experience, when comparing the gene expression profiles of a tissue under two different conditions, only a minority of transcripts display statistically significant differential expression. Accordingly, I would not have expected most genes involved with NFκB signaling to be differentially expressed in peripheral blood mononuclear cells (PBMCs) of patients having a non-blood disease such as RCC as compared to PBMCs of disease-free humans.

5. To confirm this, I have re-analyzed the Affymetrix gene chip profiles generated during the studies included in the present patent application for RCC and healthy PBMCs to determine whether the RCC-specific signatures in PBMC from RCC patients reflect a general NFκB activation signature or exhibit specificity where only certain genes related to NFκB activation are differentially expressed. Specifically, I assessed the expression levels of a set of genes identified in the literature as NFκB target genes (Feuerhake *et al.* (2005) Blood 106(4):1392-1399). A total of 83 qualifiers from the Affymetrix HgU95Av2 gene chip were identified and analyzed. Of the 83 qualifiers corresponding to transcripts modulated by NFκB activation, 55 probesets were called present in 1 or more samples with expression levels where frequency was equal to or greater than 10 ppm in one or more samples. Of these 55 probesets, only 6 probesets corresponding to 4 genes (BL2-like 1, superoxide dismutase 2, MCP1 and IL6) were altered at least 2-fold with a statistical significance in a t-test of less than 0.05 (unadjusted). The remaining 49 NFκB signature probesets failed to meet these criteria designed to establish disease-associated transcripts. These data strongly suggest that only a portion of the NFκB signature, along with

alterations in transcripts in many other pathways, contributes to the overall transcriptional pattern in PBMCs responsible for the differences in RCC patients and disease-free control subjects.

6. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present application or any patent issued in reliance thereon.

Date: 9/14/06

Michael E. Burczynski, Ph.D.